REMARKS

Claims 1-5, 10-19, 30, and 36-41 are pending in this application, with claim 1 being independent. By this Amendment, claims 1-5, 10-19 and 30 have been amended, claims 6-9, 20-29, and 31-35 have been cancelled without prejudice to or disclaimer of the subject matter contained therein, and claims 36-41 have been newly added. Applicants expressly reserve the right to pursue the subject matter of the cancelled claims in a divisional application. No new matter has been added by this Amendment.

Applicants' Claimed Invention

The presently-claimed invention relates to a method for determination of amounts or relative proportions of more than one individual polynucleotide sequence or subgroups thereof in a sample including a mixture of target ribopolynucleotide sequences using a quantitative affinity aided solution hybridization in combination with size- or mass-based fractionation. The method comprises the consecutive steps of:

(a) providing, one or more pools with more than one soluble polynucleotide probe, wherein each probe in said pool is complementary to an individual target ribopolynucleotide sequence in the sample, being present in a molar excess as compared to the target ribopolynucleotide sequences, and has approximately the same number of hybridizing nucleotides, which are complementary to said target ribopolynucleotide sequences, wherein approximately the same number of nucleotides means that the polynucleotide probes are not distinguishable from each other in size- or mass-based separation, fractionation and recording and are made distinguishable by providing said polynucleotide probes with one or more resolution enabling tags, which tags are oligonucleotide residues, which change the mass or size of the polynuclotide

probes and provide them with different mobilities in fractionation, separation or recording systems without disturbing the hybridization or capturing reaction, wherein each pool of polynucleotide probes are placed in their own vessels;

(b) providing a mixture of affinity tagged target ribopolynucleotide sequences by contacting the sample comprising the mixture of target ribopolynucleotide sequences with at least one affinity tag; and thereafter

(c) performing steps (i) and (ii) simultaneously, or sequentially; in the order (i) and (ii), wherein steps (i) and (ii) comprise: (i) allowing a hybridization reaction to take place between the molar excess of polynucleotide probes from step (a) and the affinity tagged target ribopolynucleotide sequences from step (b) leading to formation of hybrids; (ii) providing captured hybrids by recovering the hybrids, on a separation aiding tool provided with an affinity pair of the affinity tag of the target ribopolynucleotide sequences;

(d) providing released polynucleotide probes by eluting the polynucleotide probes in an unmodified form from the captured hybrids, wherein said released polynucleotide probes are provided with tracer tags in step (a) or are tracer tagged after release in step (d) or during or after amplifiecation after the release in step (d);

(e) separating the released polynucleotide probes by electrophoretic or chromatographic techniques or mass spectrometry and recording the amount or relative proportions of distinguishable polynucleotide probes, the amount of which corresponds to the amount of complementary target ribopolynucleotide sequences in the mixture of target ribopolynucleotide sequences in the sample.

Rejections under 35 U.S.C. § 112

Second Paragraph - Indefiniteness

The Office Action has rejected claims 1-35 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as their invention for the reasons set forth in paragraphs (a)-(l) found on pages 2-4 of the Office Action. Without conceding the propriety of this rejection, Applicants submit that it has been overcome by the claim amendments presented above.

Applicants have retained the term "quantitative" only in the preamble of claims. Applicants submit that the term "quantitative" is not indefinite, as the specification and claims sufficiently explain how the hybridization may be performed in a quantitative manner, which evident from the presently-claimed invention, the term "quantitative" is used in the preamble to refer to the affinity aided solutions that may be used in accordance with the methods of the presently-claimed invention, such as those set forth in Leying et al. (which bears the title "Method of Quantitatively Detecting Nucleic Acids").

Applicants have deleted the allegedly unclear definition "organized pools."

Applicants have deleted the allegedly indefinite recitation "preset optional number" and and replaced it with "more than one."

Applicants have defined the recitaition "approximately," especially the feature "having approximately the same number of hybridizing nucleotides" by adding a further definition "wherein approximately the same number of nucleotides means that the polynucleotide probes are not distinguishable from each other in size- or mass-based separation, fractionation and recording and are made distinguishable by providing said

polynucleotide probes with one or more resolution enabling tags, which tags are oligonucleotide residues, which change the mass or size of the polynuclotide probes and provide them with different mobilities in fractionation, separation or recording systems." The new definition is supported at least by original claim 19, and the definition found in the specification on page 10, last paragraph.

Applicants have amended the feature "the affinity pair," which was rejected for allegedly lacking antecedent basis, by replacing it with the recitation "an affinity pair of the affinity tag."

Applicants have deleted the allegedly unclear recitations "dynamic" and "more or less" in claims 2 and 4.

In claim 4, the term "deoxyribonucleotide," which was rejected as allegedly lacking antecedent basis, has been replaced by the term "polynuclotide."

Claim 11 has been amended by transferring the recitation "at least one" into claim 1, maintaining the recitation "more than five" in claim 11, and transferring the recitation "more than ten" into new claim 37.

Claim 14, which was rejected for allegedly having insufficient antecedent basis for "primer," has been amended to positively recite the term.

Claims 19 and 30 have been amended, and claims 20-29 and 31-35 have been cancelled. Therefore, these rejections are moot.

Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection of claims 1-35 under 35 U.S.C. § 112, second paragraph.

First Paragraph – Utility and Enablement

The Office Action has rejected claims 19-35 under 35 U.S.C. § 112, first paragraph, as allegedly not being supported by a specific or well-established utility, and on the grounds that one skilled in the art would not know how to use the claimed invention, which Applicants have interpreted as a rejection based on an alleged lack of enablement. Applicants respectfully traverse these rejections.

Applicants submit that these rejections have been overcome by the above amendments to present claim 19, which has been amended to clarify that the method steps are performed on a test kit.

Claims 20-29 and 31-35 have been cancelled without prejudice to or disclaimer of the subject matter contained therein.

Claim 30 now refers to the method and indicates that the pools comprising the polynucleotide probes are placed in wells of microtiter plates.

For at least the above reasons, Applicants respectfully request reconsideration and withdrawal of the rejection of claims 19-35 under 35 U.S.C. § 112, first paragraph.

Rejections under 35 U.S.C. § 103

Rejection over Leying et al. in view of Grossman et al.

Claims 1, 5-13, 16, 19, 21, and 23-31 were rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Leying et al. (U.S. Patent No. 6,136,531) in view of Grossman et al. (U.S. Patent No. 5,807,682).

Initially, Applicants submit that even though the abstract of Leying et al. indicates that the patent discloses a method of quantitatively detecting specific nucleotide sequences via solution hybridization and subsequent immobilization of hybrids on a

solid phase, Leying et al. only discloses the quantification of one type of target sequence per assay. This is clearly specified in Leying et al., *see e.g.* the Abstract. Leying et al. does not disclose methods for determining the amounts or relative proportions of more than one individual ribopolynucleotide sequence or subgroups thereof in a sample.

Leying et al. discloses a method of immobilizing the target sequence to allow for removal of excess probe, which is a basic problem in hybridization assays. However, the method of Leying et al. is performed by solution hybridization between a mixture of target mRNA and one probe per hybridization vessel. In contrast to the presently-claimed invention in which a mixture of probes is added to the mRNA mixture and forms a plurality of hybrids which are captured on a solid support, Leying et al. disclose the formation of only one type of hybrid, which is subsequently immobilized on a solid phase. Even if Leying et al. describe two types of probes, they are not used in a mixture. Furthermore, the probes of Leying et al. do not have the same size, and even if they are labeled they are never released from the solid support. The incorporated tracer is recorded by adding anti-DIG-POD antibody and the reagent luminol/iodophenol, not by releasing the probe.

The Office Action admits that Leying et al. does not disclose probes that have approximately the same number of hybridizing nucleotides, quantitatively releasing probes in unmodified form from the hybrids captured, or the step of separating and recording the amount or relative proportions of probes wherein the probes correspond to the amount of target RNA in the sample. However, the Office Action takes the position that these missing features are allegedly taught by Grossman et al.

Like Leying et al., Grossman et al. also does not disclose methods for determining the amounts or relative proportions of more than one individual ribopolynucleotide sequence or subgroups thereof in a sample.

Applicants agree that Grossman et al. discloses the use of a mixture of probes having the same length. Even if Grossman et al. discloses release of such probes, the probes are not released in unmodified form. The probes may be ligated, as in the preferred embodiment of Grossman et al. (*see* column 15, lines 36-42), and they must be provided with long polymer chains before or after the hybridization while the probe is annealed to the target as a hybrid.

From column 15, lines 36-45, it is also evident that Grossman et al. never contemplates quantification, despite the peaks in Figures 6, 9, and 11, and the method is intended solely for qualitative detection. The long polymer chains would disturb the hybridization and capturing reactions required for quantification. Furthermore, Grossman et al. do not disclose that any capillary electrophoresis method can be used to fractionate probes of similar sizes and in similar conditions. Grossman et al. disclose that probes of similar length have to be fractionated in a non-sieving medium and that in order to enable separation of the individual probe they have to be provided with several polymer chains changing the charge frictional drag. Grossman et al. accordingly fails to disclose or suggest that by measuring the probes the targets can be quantified.

Further, in the method of Grossman et al., all probes which have hybridized to the target are chemically modified, while the unreacted probes are not. The modification is performed by ligation (method A), by PCR (method B), by polymerase extension (method C) or by cleavage (method D). Hence, the need to remove unreacted probe is less critical, but the added chemical steps decrease the level of

quantitativeness. Ligation and cleavage are, for instance, very ineffective on DNA-RNA hybrids. The method depicted in Figure 9 of Grossman, which is performed on DNA, would be non-quantitative if performed on RNA.

In addition, Grossman et al. only discloses DNA targets, whereas the presently-claimed invention is related to determining the amount of RNA, which is known to be unstable. Applicants note that the conditions used in Grossman et al. include enzymatic reactions which require the use of purified nucleic acids. Under these conditions, any RNA in solution would be degraded. Further, even if mRNA were to be attached to a solid support, which Grossman et al. does not disclose, there would be steric hindrances.

Applicants therefore submit that the combination of Leying et al. and Grossman et al. fails to disclose or suggest the features of the presently-claimed invention set forth above. Applicants also submit that one skilled in the art would not combine Leying et al. and Grossman et al. in order to arrive at the presently-claimed invention without the benefit of improper hindsight reconstruction based on Applicants' disclosure, because doing so would render the method of quantitatively detecting specific nucleotide sequences of Leying et al. unsuitable for use in the presently-claimed methods of determining the amounts or relative proportions of more than one individual ribopolynucleotide sequence or subgroups thereof in a sample.

Accordingly, for at least these reasons, Applicants submit that claims 1, 5-13, 16, and 19, 21, and 23-31 are not unpatentable over Leying et al. in view of Grossman et al., and respectfully request that this rejection be withdrawn.

Rejection over Leying et al. in view of Grossman et al., further in view of Chen-Liu et al.

Claims 14 and 15 were rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Leying et al. in view of Grossman et al., further in view of Chen-Liu et al. (*Genomics* 30: 388-392 (1995)).

The deficiencies of the combination of Leying et al. and Grossman et al. are discussed above. Further, the Office Action admits that Leying et al. and Grossman et al. do not teach a method wherein released probes are amplified by PCR and recorded with a recording system based in the resolution enabling tags in claim 14 and wherein the primer is a universal primer in claim 15.

Chen-Liu et al. is cited for teaching a method of using PCR to amplify a microdissected chromosome with a universal primer. Chen-Liu et al. discloses that the universal primer-mediated PCR amplification and labeling enables specific detection of the target DNA by subsequent FISH or Dot blot analyses, wherein the target sequence detection is recorded with a recording system selected based on the resolution of resolution-enabling tags. Chen-Liu et al. teaches that the method of amplifying probes results in enrichment of the target sequences (page 390, left column, line 1).

However, Applicants submit that Chen-Liu et al. fails to remedy the deficiencies of the combination of Leying et al. in view of Grossman et al. In particular, Chen-Liu et al. does not disclose or suggest methods of determining the amounts or relative proportions of more than one individual ribopolynucleotide sequence or subgroups thereof in a sample.

Instead, Chen-Liu et al. discloses preparation of a sub-library of a cDNA library in order to provide more specific detection by demonstrating that a particular region of a

chromosome, i.e. the target sequence, is amplified with a universal primer and a cDNA probe from a cDNA library, which is hybridized to a biotin-labeled target DNA and captured on magnetic beads. The cDNA is released and recovered by PCR amplification. This procedure is used to enrich targets and cDNA, and enables the selection of more specific sublibraries from a cDNA library. It provides a set of probes useful for microdissection, and enables specific detection of a target present in small amounts. However, Chen-Liu et al. does not teach quantification of targets present in small amounts by amplifying hybridized affinity captured and released probes. The detection of targets in a microsection is not a quantitative determination of the amounts of the targets.

In the presently-claimed invention, the targets are ribopolynucleotide sequences, and the targets are never amplified. The targets, which are captured on the solid support, are discarded. Only the probe is amplified if it is present in very small amounts. The released single stranded probes provided with primers are recovered and amplified. This method of quantification is more reliable because there is no need to amplify the target.

Accordingly, Applicants submit that claims 14 and 15 are not unpatentable over Leying et al. in view of Grossman et al., further in view of Chen-Liu et al., and respectfully request that this rejection be withdrawn.

Rejection over Leying et al. in view of Grossman et al., further in view of Amann et al.

Claims 2-4, 17, 20, 22, 32 and 34-35 were rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Leying et al. and Grossman et al., further in view of in view of Amann et al. (*Applied and Environ Microbiol.* 56: 1919-1925 (1990)).

The deficiencies of the combination of Leying et al. and Grossman et al. are discussed above. Further, the Office Action admits that Leying et al. and Grossman et al. do not teach a method of selecting probes from conserved or hypervariable regions from intragenic region sequences for subgroups, species subspecies of transcripts expressed in the organism in claims 2 and 20; wherein the mixed target RNA is mRNA or rRNA in claims 3 and 5; and probes are designed for determination of variations in the amounts or relative proportions of polynucleotide sequences comprising the mixed target populations in claim 4, 22; and wherein test kits of probes used to determine comparative, quantitative assessment of variations in the amounts of organisms are used in claims 17 and 32; use of a kit for assessing hygienic conditions and epidemiologic situations in claims 34 and 35.

Amman et al. is cited for teaching a method wherein probes are designed from species or group-specific ribonucleotide sequences with conserved or hypervariable regions from intragenomic rRNA sequences, where the probes are used to detect and enumerate specific cells in mixtures of cells. Amann et al. is also cited for teaching a method wherein the probes are used to hybridize to fixed cells of mixed populations thereby exposing analytes including mRNA and rRNA to the probes for specific hybridization (page 1921, left column, paragraph 1). The DNA probes may be designed from group or species specific RNA sequences that represent different phylogenetic levels, eukaryotes, eubacteria, sulfate-reducing bacteria, and *Desulfobacter* in a mixed target population (page 1920, left column, paragraph 2; page 1922, right column, paragraph 2). The probes may be used to measure total abundance of microbial species in an environment and to assess differences in cellular rRNA content (page 1919, left column, paragraph 2), permitting detection and identification of virtually any

microorganism (page 1920, left column, paragraph 1, lines 10-11) and tracking of population changes (page 1923, right column, last sentence).

However, Applicants submit that Amann et al. fails to remedy the deficiencies of the combination of Leying et al. in view of Grossman et al. Amman et al. merely discloses a method wherein probes are designed from species or group-specific ribonucleotide sequences or conserved or hypervariable regions from intragenomic rRNA sequences. The presently-claimed invention is not related to counting cells with FISH using labeled probes from ribosomal RNA. The claimed invention is a novel and unobvious method for quantifying target ribonucleotide sequences, and may be used to compare the amount of conserved or hypervariable regions in cell populations. The inventive methods may be adapted for use in studying ribosomal RNA.

Accordingly, Applicants submit that claims 2-4, 17, 20, 22, 32 and 34-35 are not unpatentable over Leying et al. in view of Grossman et al., further in view of Amann et al., and respectfully request that this rejection be withdrawn.

Rejection over Leying et al. in view of Grossman et al., further in view of Amann et al. and Strathmann

Claims 18 and 33 were rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Leying et al., Grossman et al., and Amann et al., further in view of Strathmann (U.S. Patent No. 6,480,791).

The deficiencies of the combination of Leying et al., Grossman et al., and Amann et al. are discussed above. Further, the Office Action admits that Leying et al., Grossman et al., and Amann et al. do not teach a method wherein a set of multiple test kits is provided with tracer tags each being distinguishable from the other by the emitted signal.

Strathmann is cited for teaching a method of labeling probes with fluorescent tags such as fluorescent rhodamines and quantum dots to facilitate detection, thereby facilitating detection of target nucleic acids (col. 35, lines 10-15).

However, Applicants submit that Strathmann fails to remedy the deficiencies of the combination of Leying et al. in view of Grossman et al., further in view of Amann et al., particularly in view of the amendments to the presently-claimed invention to reflect that the methods are used on a test kit. Even if Strathmann is considered to disclose test kits and probes, the specific combination of probes having the characteristics set forth in the claims are not disclosed or suggested by Strathmann.

Accordingly, Applicants submit that claims 18 and 33 are not unpatentable over Leying et al. in view of Grossman et al., further in view of Amann et al. and Strathmann, and respectfully request that this rejection be withdrawn.

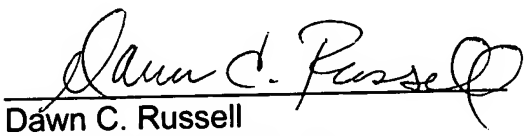
CONCLUSION

In view of the foregoing, reconsideration of the application, withdrawal of the outstanding rejections, allowance of claims 1-5, 10-19, 30, and 36-41, and the prompt issuance of a Notice of Allowance are respectfully requested.

Should the Examiner believe that anything further is necessary in order to place this application in better condition for allowance, the Examiner is requested to contact the undersigned at the telephone number listed below.

In the event that additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and any fees required therefore are hereby authorized to be charged to our Deposit Account No. 01-2300 referencing docket number **108306.00026**.

Respectfully submitted,



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Attachments: Petition for Extension of Time (2 months)
Information Disclosure Statement